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## Sensitive Genetic Analysis Reveals Vast Changes Associated with Hypertrophic Cardiomyopathy

The one-gene, one-disease concept is elegant, but incomplete. A single gene mutation can cause many other genes to start—or stop—working, and it may be these changes that ultimately cause clinical symptoms. Identifying the complete set of affected genes used to appear impossible. Not anymore.

Studying genetically modified mice, researchers led by Christine E. Seidman, a Howard Hughes Medical Institute investigator at Brigham and Women's Hospital, and her husband Jonathan G. Seidman, who is at Harvard Medical School, have identified hundreds of genes with altered expression in preclinical hypertrophic cardiomyopathy. The study, which is coauthored by colleagues at Harvard Medical School, is published in the June 9, 2007, issue of the journal *Science*. The discovery could help scientists define the pathways that lead to the disease and lead to the discovery of targets for early detection, prevention, and treatment.

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- Christine E. Seidman

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To obtain a complete picture of the genetic changes associated with the disease, the researchers developed a new gene sequencing technique called polony multiplex analysis of gene expression, or PMAGE. The technique can find messenger RNA transcripts--the directions for making a protein, spun out from the DNA of an active gene--that occur as rarely as one copy for every three cells.

To use PMAGE, researchers attach short sequences (called tags) cut from mRNAs to tiny beads. This tag is amplified, so that each bead contains millions of copies of the same mRNA tag sticking out from it like a minuscule Koosh ball. All of the beads -- now called polonies (short for polymerase chain reaction of colonies) -- are placed in one layer onto glass, and all of the tags are sequenced simultaneously. A computer program then matches the tags to known genes. The more tags associated with a gene, the higher the expression of that gene.

The industry standard for gene sequencing is serial active gene expression, or SAGE. "There are a couple of labs that have been dedicated to developing this technology," Seidman said, including HHMI investigator Bert Vogelstein at Johns Hopkins and George Church at Harvard. But PMAGE analysis costs between 1/20 and 1/9 of a comparable SAGE analysis, making it more appropriate for the kind of large-scale expression profiling undertaken in this study, she explained. "With SAGE, you can't afford to sequence 4 million transcripts."

Using PMAGE, the researchers compared a healthy group of mice to a group with a genetic mutation that causes hypertrophic cardiomyopathy (HCM) after about 25 weeks of age. In people with HCM, the heart muscle thickens and fails to relax normally after contraction. HCM is the most common cause of sudden death in athletes.

Seidman's group used cardiac tissue from 8-week-old mice to create two PMAGE libraries totaling 4.4 million mRNA tags. They found 706 genes that were overactive or underactive in HCM mice, compared with normal mice. Some genes already have been linked with HCM or heart development. Others are new to the scene.

Overactive genes included:

*Nppa* (natriuretic peptide precursor a), which encodes atrial natriuretic peptide, or ANP. This protein is an important marker for HCM.

*Ctgf* (connective tissue growth factor), *Tgfb1* (transforming growth factor beta-1), and *Postn* (periostin), powerful regulators of fibrosis and collagen deposition. The early activation of these genes indicates that fibrosis is probably a primary contributor to heart dysfunction, not a reaction to other changes.

*Vgll2* (vestigial-like 2 homolog) and *Egr3* (early growth response-3), transcriptional regulators involved in the fetal development of the heart muscle

*Nr1h3* (nuclear receptor subfamily 1, group h, member 3) and *Nfkbie* (nuclear factor kappa light polypeptide gene enhancer in B cells inhibitor epsilon), which have never before been linked with HCM

Underactive genes included:

*Hod* (homeobox-only protein) and *Hand2* (hand and neural crest derivatives 2), transcriptional regulators involved in the fetal development of heart muscle

*Abcc9* (adenosine triphosphate cassette subfamily C member 9), which encodes part of the cardiac potassium channel. This channel helps to regulate calcium balance. Mice who lack *Abcc9* develop arrhythmias and myocardial calcium overload.

*Sln* (sarcolipin) and *Pln* (phospholamban), which regulate calcium uptake into muscle cells

"It's important that we could statistically quantify changes even in genes with very low expression levels," Seidman said. "Some of these low-abundant molecules may be very important in altering cell biology in ways that may be part of the root cause, or a compensatory response to very early manifestations of disease."

Seidman is now repeating the PMAGE sequencing using tissue from younger mice. "We want to get at the drivers of the pathology," Seidman said. "HCM largely affects structural proteins, and we don't really understand how a change in one protein affects the cascade that ultimately affects physiology. If we do this sequencing early enough, we'd like to think we'll see signals that point us to something that is fundamentally changing the whole downstream cascade."

Discovering those fundamental drivers of change could result in targeted therapies to halt HCM in its tracks, or even prevent it altogether.

"We're doing this in a mouse right now, but we have access to tissue from human patients," Seidman said. "With the deep, rich analysis we obtain from a biopsy or resection, we can jump into understanding the human biology of heart disease quite quickly."